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Seasonal nitrogen uptake and regeneration in the western coastal Arctic

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Abstract

Here, we present the first study to investigate the seasonal importance of amino acid-nitrogen (N) to Arctic near shore microbial communities. We measured primary productivity and the uptake of ammonium, nitrate, urea, and amino acids in two size fractions ($> 3 \mu\text{m}$ and approximately $0.7\text{--}3 \mu\text{m}$), as well as ammonium regeneration and nitrification using ^{15}N and ^{13}C tracer approaches in the near-shore waters of the Chukchi Sea, during January, April, and August for two consecutive years. At discrete depths, nitrate comprised 46–78% of the total dissolved N pool during January and April but only 2–6% during August. Dissolved organic N (DON) concentrations increased between January and August though the carbon (C) : N (mol : mol) of the DON pool declined. Of the substrates tested, amino acids supported the bulk of both N and C nutrition in both size fractions during January and April (ice-covered). Urea generally had the lowest uptake rate under ice-covered conditions; uptake of urea-C was only detectable in August. Though previous Arctic studies focused largely on nitrate, we found nitrate uptake was generally lower than other substrates tested. The sharp decline in nitrate concentration between April and August, however, indicates a drawdown of nitrate during that period. Rates of ammonium uptake were highest in August, when it was the dominant N substrate used. During all sample periods, rates of ammonium regeneration were sufficient to supply ammonium demand. Rates of nitrification varied between sample periods, however, with much higher rates seen in January and April.

Low temperatures have historically been thought to constrain microbial activity, however, several recent studies have shown that this inhibition is not universal and that the Arctic has an abundant and well adapted community of psychrotolerant and psychrophilic (i.e., cold-loving) microorganisms (Yager et al. 2001; Hodges et al. 2005; Connelly et al. 2006). This abundant microbial activity is especially pronounced in coastal communities, where high overall production has been demonstrated (Bates et al. 2005). In the western coastal Arctic, primary and secondary production are principally supported by nitrogen (N) inputs from the Pacific Ocean via the Bering Strait. In this system, N is drawn

down to limiting levels during the summer when primary productivity is highest (Codispoti et al. 2005; Ortega-Retuerta et al. 2012). In contrast, heterotrophic bacterial communities are believed to be carbon (C) limited when a surplus supply of dissolved organic matter (DOM) is not produced by phytoplankton (Kirchman et al. 2005, 2009a). Spring inputs of riverine DOM may also provide labile material (Letscher et al. 2011), which is likely important to both primary and secondary producers (Kirchman et al. 2009b; Wickland et al. 2012). As such, the uptake of N and C are subject to intense competition by autotrophic and heterotrophic microorganisms (Fouilland et al. 2007). This balance between autotrophs and heterotrophs is a fundamental driver of ecological parameters in the Arctic marine environment and has important implications for food web dynamics and C exchange with the atmosphere.

In addition to the importance of N and C uptake in the Arctic ecosystem, the balance of these nutrients is potentially changing. The Arctic Ocean is undergoing dramatic

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Additional Supporting Information may be found in the online version of this article.

Table 1. Locations and physical parameters for sampling sites. During ice-covered seasons, sample depth was measured from the bottom of the sea ice. ND indicates no data.

| Month | Day | Year | Latitude (W) | Longitude (N) | Water depth (m) | Sample depth (m) | Ice thickness (m) | PAR* ($\mu\text{mol s}^{-1} \text{m}^{-2}$) | Temp. ($^{\circ}\text{C}$) | Salinity |
|---------|-----|------|--------------|---------------|-----------------|------------------|-------------------|---|------------------------------|----------|
| January | 26 | 2011 | 156°41'40" | 71°20'21" | 10.4 | 2.0 | 0.91 | 0.30 | -1.8 | ND |
| | 28 | | 156°41'40" | 71°20'21" | 10.4 | 2.0 | 0.91 | 0.30 | -1.8 | 32.9 |
| | 16 | 2012 | 156°41'00" | 71°21'20" | 16.5 | 2.0 | 1.20 | 0.08 | -1.8 | 33.5 |
| | 21 | | 156°34'33" | 71°22'12" | 7.0 | 1.0 | 0.56 | 0.12 | -1.8 | 33.8 |
| April | 23 | 2010 | 156°41'09" | 71°21'22" | 17.7 | 8.0 | 0.55 | 5.0 | ND | ND |
| | 25 | | 156°41'09" | 71°21'22" | 17.7 | 8.0 | 0.55 | 5.0 | ND | ND |
| | 26 | 2011 | 156°43'16" | 71°18'07" | 17.0 | 6.5 | 1.50 | 4.7 | -1.6 | 31.5 |
| | 30 | | 156°43'39" | 71°18'13" | 8.0 | 4.0 | 1.30 | 11 | -1.8 | 32.4 |
| August | 25 | 2010 | 156°41'09" | 71°21'22" | 12.5 | 4.0 | 0.0 | 65 | 6.0 | 31.4 |
| | 28 | | 156°41'09" | 71°21'22" | 12.5 | 4.0 | 0.0 | 44 | 6.0 | 31.6 |
| | 15 | 2011 | 156°43'16" | 71°18'07" | 17.0 | 4.0 | 0.0 | nd | 5.9 | 30.2 |
| | 17 | | 156°43'16" | 71°18'07" | 17.0 | 8.0 | 0.0 | nd | 4.7 | 30.6 |
| | 19 | | 156°43'39" | 71°18'13" | 8.0 | 2.0 | 0.0 | nd | 4.7 | 30.2 |

* Photosynthetically active radiation.

changes including increases in air and water temperature, melting of permafrost in the coastal zone, and declines in sea ice (Wassmann et al. 2011). As the amount of open water in the Arctic increases and freshens, primary productivity, especially along the coasts, is expected to rise as a result of enhanced light penetration (Arrigo et al. 2008; Pabi et al. 2008). If nutrient limitation is not alleviated by increased upwelling on the shelves (Carmack and Chapman 2003), increased delivery of labile terrigenous DOM, or active N fixation, primary production would be increasingly limited by N supply, thereby reducing the ability of the Arctic Ocean to act as a sink of carbon dioxide (Cai et al. 2010). A thorough understanding of N uptake and regeneration is needed to determine how the system is currently structured, and how it may react to changes in nutrient supply and removal processes brought on by the rapid changes observed and predicted in the Arctic.

Recent investigations of microbial population dynamics and productivity in the Arctic have shown that heterotrophs remain active during winter, when decoupled from phytoplankton activity (Alonso-Sáez et al. 2008; Garneau et al. 2008). The logistical difficulties of sampling in the remote Arctic environment have restricted studies of the nutrient concentrations and concomitant uptake to support growth, especially over a full seasonal cycle, although there are some notable exceptions in the Canadian Arctic (Simpson et al. 2008, 2013a; Tremblay et al. 2008; Martin et al. 2012). With months of no direct sunlight, temperatures that remain well below freezing for much of the year, and an unstable sea-ice landscape subject to shifting currents and winds, the majority of studies investigating N uptake have focused on areas amenable to research vessels and have sampled during the late spring or summer months. However, the Arctic

continental shelf accounts for 20% of the global continental shelf and Arctic coastal regions with water depths 1–18 m account for 17% of Arctic shelf area. This project sought to fill the data gap by quantifying nutrient inventories and size-fractionated uptake and regeneration rates of N and C during January, April, and August in the coastal waters of the Chukchi Sea near Barrow, Alaska.

Methods

Field sample collection

Sampling was performed during three sampling periods over two successive years centered on 71°21' N, 156°41' W, which is approximately 2.5 km northwest of Barrow, Alaska (Table 1). To capture the extreme Arctic light and physical conditions, sampling took place during January (26–30 January 2011 and 16–21 January 2012), April (22–25 April 2010 and 26 April 2011–02 May 2011) and August (25–29 August 2010 and 15–20 August 2011). During January and April the sample site was covered by landfast ice; during August the site was in open water. Each of the sampling efforts included two trips to the sampling site and the data collected were averaged.

January and April sampling was accomplished by cutting through the landfast sea-ice with a 20 cm diameter auger to sample the seawater below, while August sampling was accomplished from a small boat. Instantaneous readings of photosynthetically active radiation (PAR) was measured using a LI-193 Spherical Quantum Sensor (LI-COR Biosciences, Lincoln, Nebraska); the sensor was mounted on a 0.5 m extending arm to measure light levels under the ice during January and April and to avoid shading from the boat during August. Instantaneous readings were not as variable as would be expected in more temperate locations due

to the sun's position low on or below the horizon, depending on the season. Once we accessed the water column, sample collection was performed from a tent placed above the hole. Depth profiles of temperature and salinity were measured using a hand-deployed water quality sonde. A YSI sonde was used during the first three field trips; beginning in April 2011 we used a Manta 2 multi-sonde. Both instruments were initially calibrated by, and then maintained, as recommended by the manufacturers. Internal calibrations, checked against reference standards, were conducted before each deployment. For the Manta 2, temperature accuracy was $\pm 0.1^\circ\text{C}$ and resolution was 0.01°C ; salinity accuracy was $\pm 1\%$ of reading and resolution was four digits. Depths at the sites visited during this study ranged from 7.0 m to 17.7 m with rate measurements done at a single depth each day in waters ranging from 1–2 m in January, 4–8 m in April, and 2–8 m in August (Table 1). Water samples for analysis of depth profiles of chlorophyll *a* (Chl *a*), bacterial abundance and a suite of ambient nutrients were collected using a hand-deployed 1 L Niskin bottle on a metered rope. These discrete samples were taken to characterize the water column and provide context for the rate measurements. Larger volumes necessary for studies of N uptake were collected with a low-pressure submersible electric pump (Johnson Pump model #16004) powered by a portable generator. The use of the pump allowed rapid collection, thus preventing the water from changing temperature. Sampling depths were chosen that were below any fresh water inputs from surface ice melt and above any contaminating inputs from the sediments (see Table 1).

Incubations for rate measurements

Water was collected into a series of 2 L acid-washed PETG bottles. All samples were run in duplicate and were inoculated with additions of ^{15}N labeled ammonium chloride ($^{15}\text{NH}_4\text{Cl}$; 98.85% ^{15}N), potassium nitrate (K^{15}NO_3 ; 98%), dual-labeled ^{15}N - and ^{13}C -urea (98%), or a ^{15}N - and ^{13}C -labeled algal amino acid mixture comprised of 16 amino acids (96–99%; Cambridge Isotope Laboratories, Andover, Massachusetts). Labeled bicarbonate ($\text{H}^{13}\text{CO}_3^-$; Cambridge Isotope Laboratories) was also added to the bottles that received $^{15}\text{NH}_4\text{Cl}$ and K^{15}NO_3 additions. Literature values were used to estimate N tracer additions (Pomeroy et al. 1990; Wheeler et al. 1997) and alkalinity was used to derive ambient HCO_3^- concentrations. During January and April, ^{15}N additions were $0.09 \mu\text{mol N L}^{-1}$, $0.4 \mu\text{mol N L}^{-1}$, $0.04 \mu\text{mol N L}^{-1}$, and $0.04 \mu\text{mol N L}^{-1}$ for NH_4^+ , NO_3^- , urea, and amino acids, respectively. During August, all incubations received $0.05 \mu\text{mol N L}^{-1}$ of the relevant substrate. HCO_3^- additions were $170 \mu\text{mol C L}^{-1}$ during every sampling trip. For all sampling periods, these additions corresponded to average atom percent enrichments of $11\% \pm 7\%$ for NH_4^+ , $11\% \pm 10\%$ for NO_3^- , $22\% \pm 11\%$ for urea, $29\% \pm 9\%$ for the amino acid mixture, and $7\% \pm 0.5\%$ for HCO_3^- . We note that

kinetic curves were run for NH_4^+ , NO_3^- , and amino acids when ambient substrate concentrations were low enough to produce reasonable curves (Bronk, unpubl. data). The uptake rates of NH_4^+ , NO_3^- , and amino acids measured with $10 \mu\text{mol N L}^{-1}$ saturating additions were only an average of $6.8\% \pm 15\%$, $24.3\% \pm 3\%$, and $41.7\% \pm 57\%$ higher respectively, than those measured with the lowest additions used.

To reduce temperature fluctuations the bottles were placed in insulated coolers, surrounded by ambient seawater, and brought to the laboratory within 2 h of collection. Separate bottles were filled in the same manner and used to track temperature at 60 s intervals with a submerged HOBO TidbiT v2 water temperature data logger (Onset Computer Corporation, Bourne, Massachusetts); fluctuations were limited to an overall average standard deviation of 0.3°C during all trips.

Samples were incubated for 24 h at ambient light and temperature conditions (Table 1) in a temperature-controlled chamber. To mimic spectral attenuation and wavelength, light levels were maintained by fluorescent lights covered with GamColor blue films (GAM Products, Los Angeles, California) and confirmed using the PAR sensor. At the termination of the incubations, the samples were filtered through $3.0 \mu\text{m}$ silver (Sterlitech Corporation) or precombusted (450°C for 2 h) Whatman GF/F (nominal pore size of $0.7 \mu\text{m}$) filters. The filters were placed in cryovials and frozen until analysis. During our first field excursion (April 2010), we used $0.2 \mu\text{m}$ silver filters instead of GF/F filters to collect a larger percentage of the bacterial community, and $5.0 \mu\text{m}$ instead of $3.0 \mu\text{m}$ silver filters. Chronic quality control problems with the $0.2 \mu\text{m}$ silver filters resulted in extremely low or negligible flow rates, which subsequently required a switch to GF/F filters for the remainder of the study. For analysis of total dissolved N (TDN) and dissolved organic carbon (DOC), a 40 mL aliquot of filtrate was poured into acid-washed and muffled glass EPA vials and immediately frozen; the remaining filtrate was poured into polypropylene tubes and frozen until analysis of the remaining nutrients.

Sample analyses

Concentrations of Chl *a* and phaeophytin were estimated from replicate samples after acetone extraction using a Turner Design Model 10-AU fluorometer (Arar and Collins 1997); detection limit of $0.025 \mu\text{g L}^{-1}$. Chlorophyll standards were derived from spinach (Sigma-Aldrich) diluted in 90% acetone. Bacterial abundance was determined from triplicate whole water fixed in the field with additions of paraformaldehyde at a final concentration of 0.2% w/v, kept for 15 min at 5°C to ensure complete fixation, and stored at -80°C for less than 3 months before laboratory analysis at the University of Georgia. Each sample was run in duplicate on a FACScalibur flow cytometer (Becton Dickinson, San Jose, California) after staining with SYBR Green (Life Technologies, Grand Island, New York) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7–2.2 μm),

and then analyzed using FlowJo software (Treestar, San Carlos, California).

Concentrations of NH_4^+ were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983), with ammonium sulfate as the primary standard, and a detection limit of $0.05 \mu\text{mol N L}^{-1}$. Duplicates of nitrate (NO_3^-), nitrite (NO_2^-), silicate (Si), and phosphate (PO_4^{3-}) were measured on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). The primary standards for NO_3^- , NO_2^- , Si, and PO_4^{3-} were potassium nitrate, sodium nitrite, potassium phosphate, and sodium silicofluoride, respectively; all analyses have a detection limit of $0.03 \mu\text{mol N}$, Si, or P L^{-1} . Urea was analyzed using the manual diacetyl monoxime method (Price and Harrison 1987), with a detection limit of $0.10 \mu\text{mol N L}^{-1}$. Concentrations of free amino acids were measured as dissolved primary amines (DPA); in marine waters where the concentrations of NH_4^+ are not high, concentrations of dissolved free amino acids (DFAA) are the same as the concentration of DPAs (Keil and Kirchman 1991). DPAs were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984) with glycine as the primary standard, and a detection limit of $0.025 \mu\text{mol N L}^{-1}$. Measurements of TDN and DOC were made in triplicate by high temperature combustion on a Shimadzu TOC-V TNM (Hansell 1993; Sharp et al. 2002). Instrument calibration was assessed by the inclusion of deep-sea reference water samples, as provided by the consensus reference material program at the University of Miami (<http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html>). Dissolved organic N (DON) was calculated by subtracting inorganic N (NH_4^+ , NO_3^- , and NO_2^-) from TDN; the errors for all the terms were propagated to provide the standard error for DON (Bronk et al. 2000).

Isotopic measurements for ^{15}N and ^{13}C uptake rate samples were run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler. N uptake rates were calculated as per Dugdale and Goering (1967), and C uptake rates as per Hama et al. (1983). The uptake rates associated with the GF/F (nominal pore size of $0.7 \mu\text{m}$)– $3.0 \mu\text{m}$ size fraction were estimated by subtraction of the $>3.0 \mu\text{m}$ fraction in rates measured from whole water collected on GF/F filters. At the end of the incubations, NH_4^+ was isolated by solid phase extraction (Dudek et al. 1986; Brzezinski 1987). This allowed for correction of NH_4^+ uptake rates due to isotope dilution (Glibert et al. 1982) and to calculate rates of NH_4^+ regeneration and nitrification. With the exception of outliers during April 2011, when corrected for isotope dilution NH_4^+ uptake rates increased by $23\% \pm 16\%$ over the uncorrected rate. We also collected samples for isolation of the NO_3^- pool, which also included NO_2^- using the denitrifier method (Sigman et al. 2001). As a result, the nitrification rates calculated represent NH_4^+ and NO_2^- oxidation combined. We chose not to correct NO_3^- uptake rates for isotope dilution. Isotope dilution is generally negligible when ambient concentrations are

high such as those we measured during the January and April sampling periods (Table 2). Concentrations of NO_3^- were low during August, however, so we isolated the NO_3^- pool in August 2011, but found that isotope dilution corrected rates were not statistically different from the uncorrected rates.

Statistics

Data analyses were conducted in the open-source statistical software program R, version 2.15.0 for Mac (R Core Team 2012). Mean rate estimates were compared using two-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Method at the 95% confidence level. Correlation coefficients (R values) ≥ 0.4 were considered significant. To determine how the planktonic community responded to the different substrates during the different sample periods (Mccarthy et al. 1977), a relative preference index (RPI) was calculated according to the following formula:

$$\frac{\rho\text{N}/\sum \rho\text{N}}{[\text{N}]/\sum [\text{N}]}$$

where ρN is absolute uptake of a substrate and $[\text{N}]$ is the concentration of that substrate. Each is divided by the total (\sum) absolute uptake and concentration of all N substrates respectively.

Results

Based on the distinct physiochemical differences between sampling periods (January, April, and August; Supporting Information Fig. S1), results have been analyzed by sampling period, and subsequently compared across all sample periods.

Physical conditions

During January 2011, sampling took place 26–28 January, the week after the first sunrise of the season. In January 2012, sampling occurred 16–21 January, during the week of first sunrise. Ice thickness was approximately 1.0 m both years and irradiance in the water column was $\leq 0.30 \mu\text{mol s}^{-1} \text{m}^{-2}$ (Table 1). The water column was well mixed, as indicated by constant temperature and salinity in the depth profiles (data not shown).

During the April sampling trips, the site experienced over 20 h d^{-1} of full sun. Sea ice thickness averaged 1.0 m. As observed during the January, the water column appeared to be well mixed with respect to temperature and salinity. At the sampling depth used for all rate measurements, PAR was approximately $5 \mu\text{mol s}^{-1} \text{m}^{-2}$ with the exception of our final April trip in 2011, when the PAR was $11 \mu\text{mol s}^{-1} \text{m}^{-2}$, likely because we sampled at a shallower depth (Table 1).

During the August sampling trips the sampling site experienced 17–19 h d^{-1} of sunlight, and as in other periods the water column was well mixed with respect to temperature ($5.5 \pm 0.7^\circ\text{C}$) and salinity (30.7 ± 0.6). At the depth of

Table 2. Ambient biological and chemical inventories. The mean and standard deviation of Chl *a*, bacterial abundance, and nutrient concentrations are given for the depth where rate measurements were made (see Table 1) during each trip, which consisted of *n* visits to the site.

| Month | Year | <i>n</i> | Chl <i>a</i> ($\mu\text{g L}^{-1}$) | Bacterial abundance (10^8 cells L^{-1}) | Particulate N ($\mu\text{mol N L}^{-1}$) | NH_4^+ ($\mu\text{mol N L}^{-1}$) | NO_3^- ($\mu\text{mol N L}^{-1}$) | Urea ($\mu\text{mol N L}^{-1}$) | DPA* ($\mu\text{mol N L}^{-1}$) | PO_4^{3-} ($\mu\text{mol P L}^{-1}$) | Si ($\mu\text{mol Si L}^{-1}$) | DOC ($\mu\text{mol C L}^{-1}$) | DON ($\mu\text{mol N L}^{-1}$) |
|---------|------|----------|--|--|---|---|---|--------------------------------------|--------------------------------------|--|-------------------------------------|-------------------------------------|-------------------------------------|
| January | 2011 | 3 | 0.03 ± 0.00 | 3.0 ± 0.4 | 0.50 ± 0.01 | 2.45 ± 0.60 | 6.93 ± 1.03 | 0.25 ± 0.05 | 0.14 ± 0.04 | 1.35 ± 0.08 | 20.0 ± 2.9 | 75.3 ± 2.24 | 4.31 ± 0.18 |
| | 2012 | 3 | 0.02 ± 0.01 | 2.8 ± 0.3 | 0.87 ± 0.74 | 0.69 ± 0.24 | 9.07 ± 3.11 | 0.17 ± 0.04 | 0.07 ± 0.01 | 1.28 ± 0.19 | 28.4 ± 8.3 | 83.1 ± 1.81 | 3.05 ± 0.42 |
| April | 2010 | 2 | 1.01 ± 0.52 | 0.9 ± 0.1 | 0.25 ± 0.09 | 0.69 ± 0.11 | 5.91 ± 1.25 | 0.38 ± 0.39 | 0.15 ± 0.14 | 0.98 ± 0.04 | 10.8 ± 1.3 | 90.4 ± 7.31 | 5.16 ± 1.09 |
| | 2011 | 3 | 0.09 ± 0.03 | 3.2 ± 0.9 | 0.43 ± 0.10 | 0.86 ± 0.37 | 9.46 ± 1.69 | 0.20 ± 0.01 | 0.10 ± 0.02 | 1.15 ± 0.17 | 34.0 ± 7.3 | 67.6 ± 0.33 | 4.40 ± 0.24 |
| August | 2010 | 3 | 1.38 ± 1.17 | 5.9 ± 3.8 | 2.4 ± 0.48 | 0.37 ± 0.11 | 0.16 ± 0.04 | 0.35 ± 0.16 | 0.20 ± 0.07 | 0.59 ± 0.04 | 5.14 ± 1.1 | 96.5 ± 3.95 | 6.06 ± 0.52 |
| | 2011 | 4 | 0.52 ± 0.18 | 19.7 ± 14.7 | 2.5 ± 0.53 | 0.80 ± 0.50 | 0.35 ± 0.07 | 0.49 ± 0.33 | 0.26 ± 0.15 | 0.52 ± 0.04 | 6.46 ± 1.9 | 88.2 ± 6.55 | 7.12 ± 1.47 |

* Dissolved primary amines.

sampling for the N uptake rate studies PAR was over $44 \mu\text{mol s}^{-1} \text{m}^{-2}$ in 2010, an order of magnitude higher than observed in April.

Ambient biological abundance and nutrient concentrations

Biological inventories were low but variable during January and April (Table 2). In contrast, concentrations of NH_4^+ ($0.7\text{--}2.4 \mu\text{mol N L}^{-1}$) and NO_3^- ($5.9\text{--}9.5 \mu\text{mol N L}^{-1}$) were generally high during those periods; NO_3^- accounted for 50–64% of TDN. January 2012 was the only trip when NO_2^- was measurable, and even then it was just barely above the detection limit ($0.03 \mu\text{mol N L}^{-1}$; data not shown). DON concentrations were lowest during January ($3.0\text{--}5.2 \mu\text{mol N L}^{-1}$), and the DOC : DON ratio ranged from 17.5 to 26.8, well above the mean stoichiometric ratio for plankton (Redfield 1934). DON was primarily composed of unidentified compounds with urea and DPA contributing less than 11% of the total DON pool.

During August, Chl *a* concentrations ($0.5\text{--}1.4 \mu\text{g L}^{-1}$) and bacterial cell counts ($5.9\text{--}19.7 \times 10^8$ cells L^{-1}) increased, while concentrations of NO_3^- were an order of magnitude lower relative to the preceding January and April (Table 2). In contrast, DON was higher during August ($6.6 \pm 0.8 \mu\text{mol N L}^{-1}$) than the other two periods. Concentrations of organic nutrients generally increased, but the DOC : DON ratio decreased to 12.2–16.0. Concentrations of both PO_4^{3-} and Si dropped during August relative to the ice-covered periods; PO_4^{3-} was <0.59 compared to $>0.98 \mu\text{mol P L}^{-1}$ and Si was <0.05 compared to $0.8\text{--}34.0 \mu\text{mol Si L}^{-1}$.

Uptake rates

Bacterial abundance was measured using flow cytometry before and after filtering through the GF/F filters, as they are known to capture some variable fraction of the bacteria (discussed in Bradley et al. 2010) as well as phytoplankton cells (Kirchman and Wheeler 1998). Our check on the size fractionation technique revealed that a potentially large fraction of bacteria were retained on the GF/F filters, and the percentages of bacterial cells retained differed depending on the sample period. During August, 61% of the bacterial cells were retained, and even more (74%) during April, while January apparently shifted to smaller cells or less aggregates and the GF/F filters only retained 36% of the bacterial cells. Thus, our N and C uptake rates include variable contributions from heterotrophic bacteria. During September in the surface waters of a Baffin Bay polynya, Fouilland et al. (2007) calculated that GF/F filters captured 80% of the total living bacterial community, which roughly aligns with our results for August.

We calculated two types of uptake rates: absolute and specific. Absolute rates are expressed in $\text{nmol N L}^{-1} \text{h}^{-1}$ and measure accumulation of a substrate within particles. Specific uptake rates are the atom percent enrichment of the cells divided by the atom percent enrichment of the

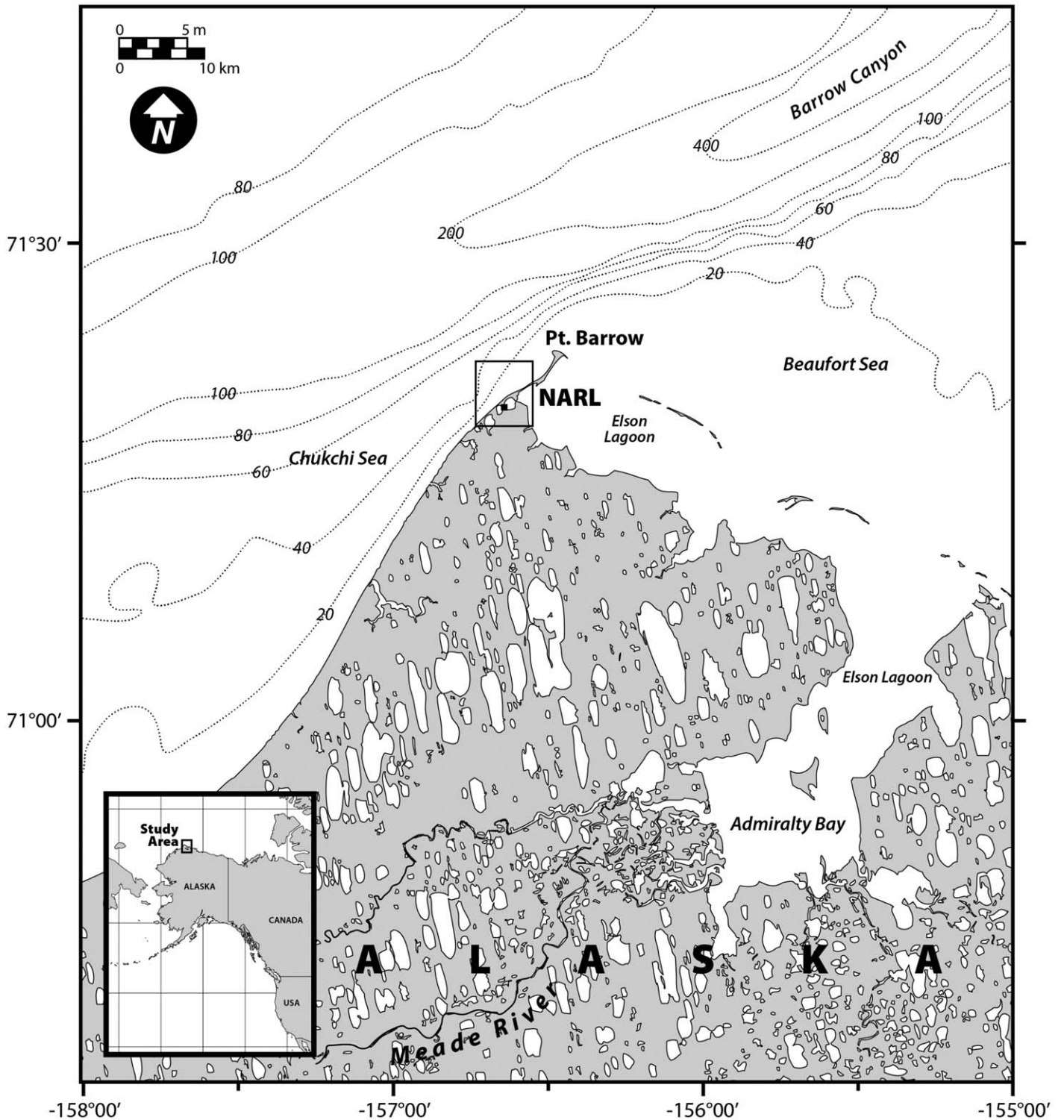


Fig. 1. Map showing the research area. Bathymetry lines are shown at 20 m intervals up to 100 m. All sample sites were located within the box, north to northwest of the Navy Arctic Research Laboratory (NARL).

substrate source pool, multiplied by time; specific uptake rates are expressed in units of per time (h^{-1}) and are analogous to growth rates (Supporting Information Fig. S2). In

January, absolute N uptake rates were generally the lowest observed across the three periods studied (Fig. 2; Table 3). Specific uptake rates exhibit the same general pattern as

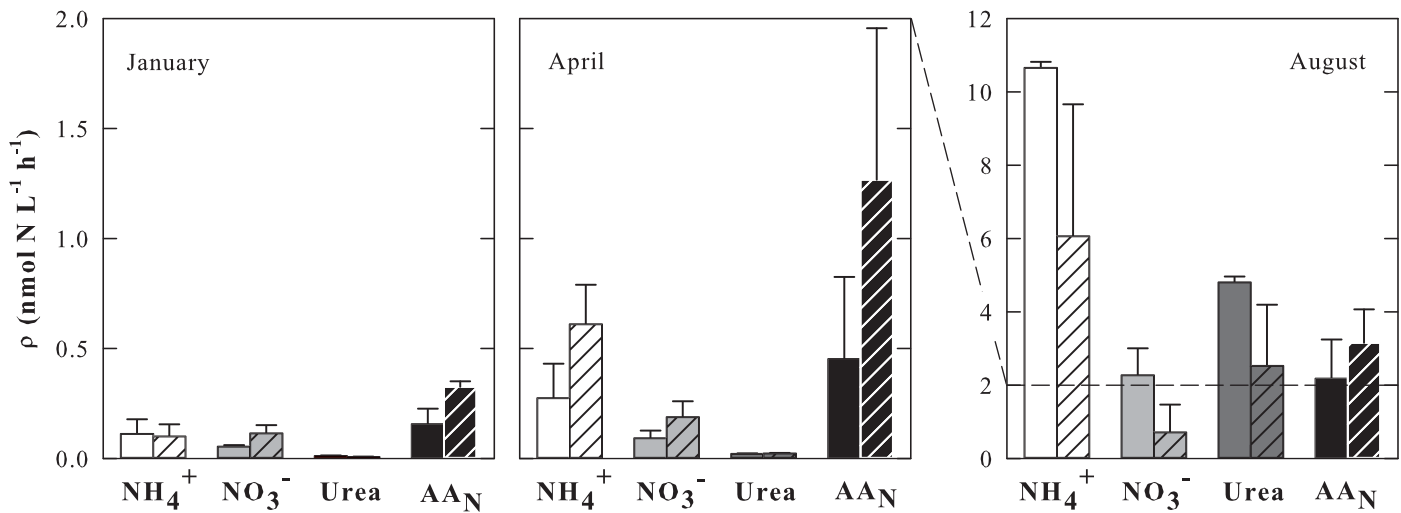


Fig. 2. Absolute uptake rates of nitrogen. Mean rates of nitrogen uptake (two experiments for each of 2 yr) for each substrate tested, with error bars representing the standard deviation ($n = 4$). Solid bars are the $>3.0 \mu\text{m}$ fraction, and hatched bars are GF/F (nominal pore size of $0.7 \mu\text{m}$) – $3.0 \mu\text{m}$ fraction. The dashed line in the August panel corresponds to the uppermost y -axis scale of the January and April panels, and is included solely for a reference.

Table 3. Absolute uptake rates. Mean and standard deviations of nitrogen and carbon uptake rates measured in the large ($> 3.0 \mu\text{m}$) and small (GF/F, nominal pore size of $0.7 \mu\text{m}$, to $3.0 \mu\text{m}$) size fractions. Note that $0.2 \mu\text{m}$ and $5.0 \mu\text{m}$ silver filters were used during the first sampling trip (April 2010), but were discontinued due to difficulty obtaining quality filters. BD is below detection.

| Month | Year | n | Size fraction | NH_4^+ ($\text{nmol N L}^{-1} \text{h}^{-1}$) | NO_3^- ($\text{nmol N L}^{-1} \text{h}^{-1}$) | Urea ($\text{nmol N L}^{-1} \text{h}^{-1}$) | AA_N^* ($\text{nmol N L}^{-1} \text{h}^{-1}$) | AA_C^* ($\text{nmol C L}^{-1} \text{h}^{-1}$) | HCO_3^- ($\text{nmol C L}^{-1} \text{h}^{-1}$) |
|---------|------|-----|-----------------------------|---|---|--|--|--|--|
| January | 2011 | 2 | $>3 \mu\text{m}$ | 0.16 ± 0.01 | 0.05 ± 0.01 | 0.01 ± 0.00 | 0.21 ± 0.14 | 0.57 ± 0.46 | BD |
| | | | GF/F- $3 \mu\text{m}$ | 0.14 ± 0.09 | 0.09 ± 0.01 | 0.01 ± 0.00 | 0.31 ± 0.13 | 0.95 ± 0.29 | BD |
| | 2012 | 3 | $>3 \mu\text{m}$ | 0.07 ± 0.00 | 0.06 ± 0.02 | 0.01 ± 0.01 | 0.11 ± 0.09 | 0.08 ± 0.11 | BD |
| April | 2010 | 2 | $>5 \mu\text{m}$ | 0.36 ± 0.00 | 0.09 ± 0.07 | 0.18 ± 0.18 | 0.11 ± 0.13 | 0.33 ± 0.42 | 0.71 ± 0.62 |
| | | | $0.2\text{--}5 \mu\text{m}$ | 0.20 ± 0.67 | 0.01 ± 0.02 | 0.06 ± 0.07 | 0.09 ± 0.02 | 0.47 ± 0.67 | 0.27 ± 0.45 |
| | 2011 | 2 | $>3 \mu\text{m}$ | 0.28 ± 0.16 | 0.09 ± 0.04 | 0.02 ± 0.00 | 0.45 ± 0.37 | 0.62 ± 0.53 | 0.13 ± 0.15 |
| | | | GF/F- $3 \mu\text{m}$ | 0.61 ± 0.18 | 0.19 ± 0.07 | 0.02 ± 0.00 | 1.27 ± 0.69 | 2.01 ± 1.09 | 0.14 ± 0.03 |
| August | 2010 | 2 | $>3 \mu\text{m}$ | 10.7 ± 3.70 | 2.79 ± 0.06 | 4.92 ± 2.79 | 1.42 ± 0.15 | 2.26 ± 0.83 | 20.4 ± 0.36 |
| | | | GF/F- $3 \mu\text{m}$ | 8.61 ± 6.56 | 1.25 ± 0.80 | 3.71 ± 0.83 | 3.80 ± 1.65 | 7.25 ± 10.3 | 4.42 ± 6.25 |
| | 2011 | 3 | $>3 \mu\text{m}$ | 10.5 ± 8.07 | 1.75 ± 1.48 | 4.69 ± 4.89 | 2.93 ± 1.58 | 9.95 ± 6.59 | 42.3 ± 25.7 |
| | | | GF/F- $3 \mu\text{m}$ | 3.52 ± 4.22 | 0.17 ± 0.15 | 1.34 ± 1.89 | 2.51 ± 1.31 | 4.26 ± 6.77 | 4.47 ± 2.92 |

* AA_N and AA_C refers to uptake rates of the nitrogen and carbon component of free amino acids respectively.

observed in absolute rates, indicating the low absolute rates were a function of both cell physiology and the relatively low biomass present. In January, there was no significant difference ($p > 0.05$) between the $>3 \mu\text{m}$ and GF/F – $3.0 \mu\text{m}$ uptake rates of NH_4^+ (Table 3). Though NO_3^- concentrations were high in January, total NO_3^- uptake was generally lower than NH_4^+ ; the GF/F – $3.0 \mu\text{m}$ fraction had higher rates than the larger ($> 3.0 \mu\text{m}$) fraction. Urea uptake rates were lower than the other substrates. Uptake rates of free amino acid N

(AA_N) were highest of all the substrates measured in both size fractions, with uptake rates in the smaller size fraction again higher than those in the larger. The amino acid mixture also appears to be a source of C, particularly for the smaller size fraction (Fig. 3; Table 3). Uptake of C from urea (Urea_C) and HCO_3^- uptake was not detected in any of the January samples.

In April, uptake rates tended to be greater than the January rates (Fig. 2). There were no statistically significant

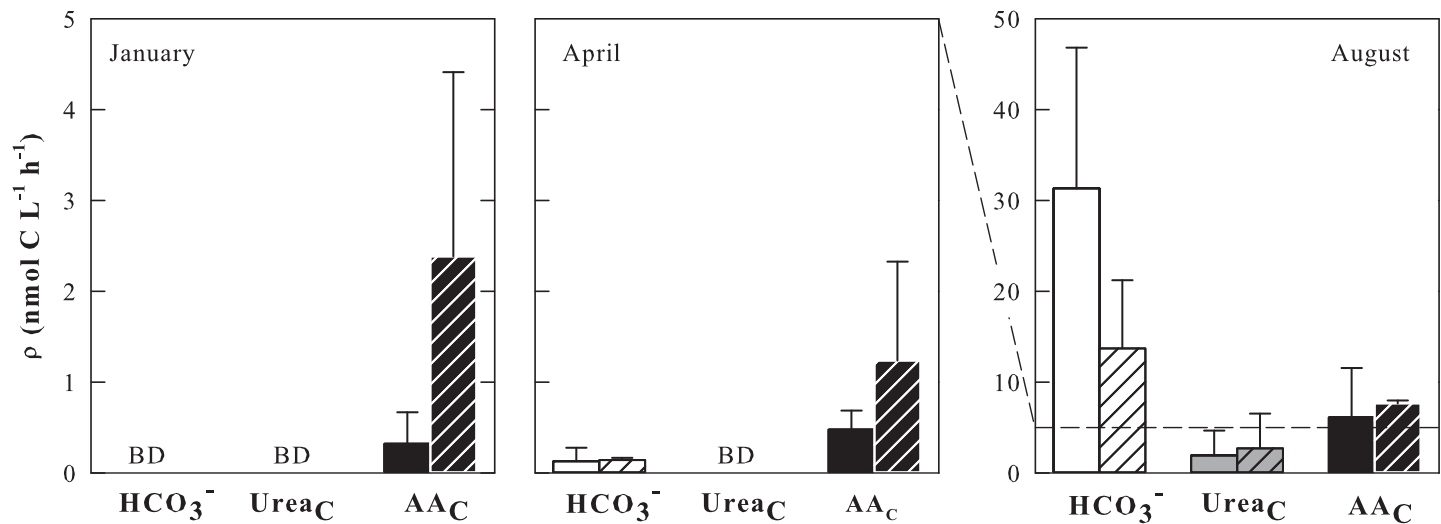


Fig. 3. Absolute uptake rates of carbon. Mean rates of carbon uptake (two experiments for each of 2 yr) for each substrate tested, with error bars representing the standard deviation ($n = 4$). Solid bars are the $> 3.0 \mu\text{m}$ fraction, and hatched bars are GF/F (nominal pore size of $0.7 \mu\text{m}$) – $3.0 \mu\text{m}$ fraction. The dashed line in the August panel corresponds to the uppermost y -axis scale of the January and April panels, and is included solely for a reference. BD indicates below detection.

differences ($p > 0.05$) in the rates between the size fractions for any of the substrates. During April, NH_4^+ continued to be an important N source. As in January, even though NO_3^- concentrations were high, uptake rates were still relatively low at $< 0.2 \text{ nmol N L}^{-1} \text{ h}^{-1}$. Free amino acids remained the preferred form of N in April, especially for the smaller size fraction, which had AA_N uptake rates approximately three times higher than the larger. Uptake rates of HCO_3^- were small but measurable and not significantly different ($p > 0.05$) between the size fractions.

During August, absolute uptake rates for all substrates were generally more than an order of magnitude higher than the other two periods, with NH_4^+ utilization being the highest relative to other substrates (Fig. 2). Unlike the ice covered periods, the larger size fraction generally had higher uptake rates. The uptake of NH_4^+ dominated both the smaller and larger size fractions. Uptake of NO_3^- in the $> 3.0 \mu\text{m}$ size fraction jumped two orders of magnitude, while the GF/F – $3.0 \mu\text{m}$ fraction also increased by the same magnitude, but only during 2010 (Table 3). Rates of urea and HCO_3^- uptake also increased by more than an order of magnitude during August. Compared to April, AA_C uptake rates in August increased by a factor of 2–16. Urea_C utilization was only detected during the August 2010 sampling trip (Table 3).

Rates of NH_4^+ regeneration averaged $13.4 \pm 7.4 \text{ nmol N L}^{-1} \text{ h}^{-1}$ for all measurements taken, but with large interannual variability during April (Table 4). Nitrification was high during the January and April periods (mean of $21.7 \pm 10.9 \text{ nmol N L}^{-1} \text{ h}^{-1}$), but dropped more than an order of magnitude during August (mean of $1.0 \pm 0.3 \text{ nmol N L}^{-1} \text{ h}^{-1}$).

We used the calculated RPI to define which substrates were taken up preferentially relative to their availability

Table 4. NH_4^+ regeneration rates and nitrification rates. Rates listed are the mean and standard deviation for each sampling trip ($n = 2$ for each row in the table). ND indicates no data.

| Month | Year | NH_4^+ regeneration ($\text{nmol N L}^{-1} \text{ h}^{-1}$) | Nitrification ($\text{nmol N L}^{-1} \text{ h}^{-1}$) |
|---------|------|---|--|
| January | 2011 | 17.2 ± 5.7 | 27.4 ± 8.2 |
| | 2012 | 10.4 ± 0.5 | 15.8 ± 9.6 |
| April | 2010 | 4.8 ± 1.4 | ND |
| | 2011 | 27.8 ± 1.2 | 24.9 ± 16.6 |
| August | 2010 | 12.5 ± 9.4 | ND |
| | 2011 | 15.2 ± 3.1 | 1.0 ± 0.3 |

(McCarthy et al. 1977). In January, the RPI of AA_N in both size fractions was at least an order of magnitude higher than the other N substrates (Table 5). In April, AA_N generally had the highest RPI, but the difference among substrates was not as large. This trend continued in August, when RPI values among the substrates were generally more equal. While this method is known to be sensitive to nutrient concentration (Stolte and Riegman 1996), DPA concentrations remained low year-round, and cannot alone explain the difference in free amino acid uptake observed during the three sampling periods. Additionally, the high uptake rates and relatively unchanging DPA concentrations imply high free amino acid regeneration in the system.

Discussion

Most marine N cycling studies in the Arctic have focused on dissolved inorganic N (NH_4^+ , NO_3^-) with limited investigations of the role of DON sources such as urea and free amino

Table 5. Relative preference index of nitrogen substrates. Means are presented for each season, size fraction, and substrate tested (NH_4^+ is ammonium, NO_3^- is nitrate, and AA_N is free amino acid N). Note that 0.2 μm and 5.0 μm silver filters were used during the first sampling trip (April 2010), but were discontinued due to difficulty obtaining quality filters.

| Month | Year | NH_4^+ | | NO_3^- | | Urea | | AA_N | |
|---------|------|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|
| | | GF/F-3.0 μm | >3.0 μm | GF/F-3.0 μm | >3.0 μm | GF/F-3.0 μm | >3.0 μm | GF/F-3.0 μm | >3.0 μm |
| January | 2011 | 1.0 | 1.7 | 0.3 | 0.2 | 0.45 | 1.0 | 41 | 31 |
| | 2012 | 1.9 | 4.5 | 0.3 | 0.3 | 0.17 | 2.8 | 90 | 63 |
| April | 2010 | 10 | 5.2 | 0.02 | 0.2 | 4.6 | 7.6 | 15 | 7.3 |
| | 2011 | 3.6 | 4.0 | 0.1 | 0.1 | 0.6 | 1.4 | 78 | 71 |
| August | 2010 | 1.3 | 1.5 | 0.4 | 0.9 | 0.8 | 0.8 | 1.4 | 0.4 |
| | 2011 | 0.9 | 1.2 | 0.1 | 0.4 | 0.4 | 0.7 | 4.3 | 2.0 |

acids. Additionally, studies have generally been conducted in offshore waters during the summer (Supporting Information Table S1). This is the first study we are aware of that provides N uptake rates in Arctic near shore waters during January, April, and August. Each period presents a unique set of physiochemical conditions, and the shallow waters of our sampling site are representative of the broad transitional area between terrestrial and offshore marine environment that is prevalent in the Arctic. In addition to variations in seasonal light and ice coverage, episodic advection can change the nutrient regime rapidly on the coastal shelves (Carmack and Chapman 2003). This leads to high regional (Codispoti et al. 1991) and interannual (Kirchman et al. 2009a) variability in community production, along with dynamic winter to summer changes in community composition (Alonso-Sáez et al. 2008). Corresponding to changes in the physical and biological realms, it is expected that nutrient inventories are also highly dynamic. Biogeochemical cycles both drive biological variables and respond to changes in the system over the yearly cycle. The three sampling periods allowed us to quantify N cycling at the extremes of light and temperature present in the region, as the confounding factors of temperature, irradiance, and nutrient concentrations have historically eluded straightforward description (Smith and Harrison 1991).

Trends throughout the year

In January, NO_3^- was the most abundant N pool while NO_3^- uptake rates were consistently lower than those of NH_4^+ and AA_N . The lower NO_3^- uptake rates were likely due to the metabolic cost of intracellular reduction of NO_3^- to NH_4^+ . Free amino acids accounted for the majority of uptake for both N and C of the substrates tested, indicating that DON and DOC sources primarily supported microbial activity during January. There was uptake of all substrates by the larger size fraction, but no concomitant evidence of primary production (i.e., HCO_3^- uptake) in January. While phytoplankton can effectively utilize DON (e.g., Berman and Bronk 2003; Bronk et al. 2007), it is likely that uptake of AA_C in the larger size fraction indicates use by particle-attached

bacteria or other microheterotrophs (e.g., microflagellates, small ciliates) that may have been collected on the 3.0 μm filters. Alternatively, or additionally, free amino acid uptake could indicate mixotrophy, which has been reported in this system (Cottrell and Kirchman 2009; Seuthe et al. 2011), and would help explain cellular maintenance by phytoplankton during the dark ice-covered winter season.

In April, surface irradiance increased dramatically but ice thickness continued to grow toward its yearly apex, which likely kept the water column community light limited (Tremblay et al. 2006; Terrado et al. 2008). Uptake of NO_3^- remained low despite high NO_3^- concentrations and higher Chl *a*. Our January and April NO_3^- uptake rates are similar to the only other study of N uptake by under-ice plankton in the Chukchi Sea, which measured NO_3^- uptake with a range of 0.02–0.39 $\text{nmol N L}^{-1} \text{h}^{-1}$ in the Canada Basin in June and July (Lee et al. 2010; Supporting Information Table S1). In fact, NO_3^- utilization was low during each of the sampling trips in our study, but especially so during January and April, even though April rates of NH_4^+ and AA_N uptake increased by more than a factor of two as compared to uptake in January. This observation is consistent with earlier work that found NO_3^- uptake to be more sensitive to low temperatures than NH_4^+ (Reay et al. 1999). The drawdown of NO_3^- concentrations observed between April and August suggests that the peak in NO_3^- uptake likely occurred between our April and August sampling trips.

Uptake rates between ice-free and ice-covered conditions can change quickly and the difference can be large (Garneau et al. 2007). When sea ice-imposed light limitation was completely lifted in the summer, the biological community responded with increased biomass and broad usage of all the N substrates measured (Table 3). The observed rapid NO_3^- drawdown and production of DON between our April and August expeditions, coupled with increases in Chl *a* and NO_3^- uptake rates, indicate high levels of productivity over the summer. As shown by the RPI results (Table 5), while AA_N was the dominant preferred substrate in January and April, substrate utilization during August was balanced across

Table 6. F-ratios for all seasons. F-ratios were calculated using the traditional approach ($\rho\text{NO}_3^-/(\rho\text{NO}_3^- + \rho\text{NH}_4^+)$) and with the inclusion of organic nitrogen forms ($\rho\text{NO}_3^-/(\rho\text{NO}_3^- + \rho\text{NH}_4^+ + \rho\text{Urea}_N + \rho\text{AA}_N)$). Means and standard deviations are presented for each season and size fraction. Note that 0.2 μm and 5.0 μm silver filters were used during the first sampling trip (April 2010), but were discontinued due to difficulty obtaining quality filters.

| Month | Year | Traditional | | Organic | |
|---------|------|------------------------|--------------------|------------------------|--------------------|
| | | GF/F-3.0 μm | >3.0 μm | GF/F-3.0 μm | >3.0 μm |
| January | 2011 | 0.42 \pm 0.15 | 0.18 \pm 0.11 | 0.18 \pm 0.07 | 0.08 \pm 0.06 |
| | 2012 | 0.66 \pm 0.25 | 0.46 \pm 0.08 | 0.25 \pm 0.19 | 0.25 \pm 0.10 |
| | Mean | 0.54 \pm 0.22 | 0.32 \pm 0.18 | 0.22 \pm 0.13 | 0.17 \pm 0.12 |
| April | 2010 | 0.04 | 0.04 \pm 0.06 | ND | 0.02 \pm 0.03 |
| | 2011 | 0.26 \pm 0.09 | 0.24 \pm 0.04 | 0.11 \pm 0.03 | 0.11 \pm 0.05 |
| | Mean | 0.21 \pm 0.13 | 0.16 \pm 0.12 | 0.07 \pm 0.06 | 0.08 \pm 0.06 |
| August | 2010 | 0.18 \pm 0.09 | 0.26 \pm 0.09 | 0.12 \pm 0.08 | 0.18 \pm 0.07 |
| | 2011 | 0.07 \pm 0.03 | 0.13 \pm 0.04 | 0.03 \pm 0.01 | 0.08 \pm 0.03 |
| | Mean | 0.12 \pm 0.08 | 0.19 \pm 0.09 | 0.07 \pm 0.07 | 0.13 \pm 0.07 |

all the N forms tested. The phytoplankton community was able to utilize the available light for photosynthesis and to incorporate N from NO_3^- , as the cost of reduction may be less than costs of competition for scarce free amino acid compounds. In Arctic surface waters, it has been found that absolute uptake rates of NO_3^- are generally only greater than NH_4^+ and urea uptake during a bloom period (Smith 1993; Kristiansen et al. 1994; Simpson et al. 2013a). At the subsurface chlorophyll maximum, Martin et al. (2012) found higher uptake of NO_3^- (relative to NH_4^+) from spring through late fall (Supporting Information Table S1), but calculated a declining dependence on NO_3^- as the season progressed. Our sampling bracketed the water column spring bloom period. Given the low concentrations of NO_3^- ($< 0.4 \mu\text{mol N L}^{-1}$) observed in August, it is clear that the period of NO_3^- draw-down and high Chl *a* was over (e.g., Yager et al. 2001) by the time our sampling began.

During late August of both study years, Chl *a* concentrations were relatively low ($< 2.0 \mu\text{g L}^{-1}$; Table 2) compared to temperate regions. With a 52–81% reduction in the concentration of Si from April to August, diatoms were likely prominent (Rózańska et al. 2009), although flagellates have been found in even greater abundance in this region (Horner and Schrader 1982). In addition, as light limitation was reduced with the seasonal cycle and retreat of sea ice, C uptake from AA_C and HCO_3^- uptake increased substantially relative to January and April. Both size fractions took up HCO_3^- in August, which likely indicates that there are small chemoautotrophs or photoautotrophs that passed through the 3.0 μm filters (Worden and Not 2008). Another potential source of C is Urea_C , but uptake rates of Urea_C from this study were relatively low. In a study in Baffin Bay, rates of Urea_C uptake were highest at more northerly sites ($> 77^\circ \text{N}$; Harrison et al.

1985), suggesting possible regional or latitudinal differences in Urea_C utilization.

The coastal area of the Chukchi Sea is highly productive (Bates et al. 2005), with high relative uptake rates of N that support primary and secondary production. During our August sampling, we measured total absolute N uptake (i.e., uptake of NH_4^+ , NO_3^- , and urea combined) at levels higher than those found in almost any other N uptake study in the western Arctic (Supporting Information Table S1). Studies from other regions of the western Arctic have found that NO_3^- uptake rates are generally equal to or greater than those of NH_4^+ , with more importance ascribed to “new” as opposed to “regenerated” production (cf. Dugdale and Goering 1967). In contrast, our study and others conducted in the region (Lee et al. 2007, 2012) show a strong preference for NH_4^+ over NO_3^- in the late summer. In our study, this trend continues during the January and April sampling periods as well. It is therefore unlikely that the NH_4^+ and DON preference is simply a matter of substrate availability, but rather an indication of a fundamental difference in the metabolic strategies of the plankton community in the coastal Chukchi Sea. Low specific uptake rates of NO_3^- relative to other N substrates during all sample periods (Supporting Information Fig. S2) support this fundamental metabolic difference hypothesis.

F-ratios have been used to compare the fraction of production within a system supported by new N (Eppley and Peterson 1979). The traditional calculation of the ratio was the uptake rate of NO_3^- , assumed to be the source of new N and representative of new production, divided by the sum of NH_4^+ uptake, representative of regenerated production, plus NO_3^- uptake. In this study, the traditional f-ratio calculated for the $> 3.0 \mu\text{m}$ fraction was highest in January (0.32 \pm 0.18;

Table 6). If the GF/F - 3.0 μm fraction is used, the f-ratio was highest in January (0.54 ± 0.22), and then decreased in April and again in August (0.12 ± 0.08). We note that the original concept of the ratio was developed in the open ocean where NO_3^- from the deep NO_3^- pool was distinguished from NH_4^+ regenerated in surface waters. The application of the f-ratio to coastal waters is problematic in that sources of new and regenerated N are not as easily distinguished. NO_3^- can be regenerated in surface waters during nitrification, a process observed at the sites in this study (Table 4; Baer et al. 2014). NH_4^+ can also come from terrestrial or riverine sources and therefore could also be considered new N. As a result, the f-ratios reported in Table 6 should be used with caution.

We further note that the original concept of the f-ratio only considered inorganic N sources. We now know that organic N can be an important, presumably regenerated source of N (Bronk and Steinberg 2008). We calculated what we term the organic f-ratio, which includes the uptake rate of urea and amino acids in the estimate of regenerated N uptake. These new organic f-ratios were always lower than the traditional but again the highest f-ratios were calculated in January in both the $> 3.0 \mu\text{m}$ (0.17 ± 0.12) and in the GF/F - 3.0 μm fraction (0.22 ± 0.13 ; Table 6). Once again, however, we know that terrestrial DOM flows into these coastal systems such that urea and amino acids could be a mixture of both new and regenerated sources of N. This may be why the f-ratios by sample period in this study are counter-intuitive: the dark, ice-covered January period would be expected to have the lowest f-ratio due to light limitation. The very low uptake rates could also cause disproportionately large differences in f-ratios, making interpreting true differences difficult.

Importance of DON

Many of the earliest studies of N uptake in the Arctic took place during the summer in the large polynyas surrounding Greenland and, as stated above, found that NH_4^+ and NO_3^- uptake were of similar magnitude (Supporting Information Table S1). When measured, urea uptake in the Arctic was generally a small component (mean = 15.0%; range = 4.4–24.1%) of the total uptake (*see* Supporting Information Table S1 for references). Our study found that urea accounted for 27% of total N uptake (not including AA_N , for a direct comparison to the other studies). The uptake rates reported here are above the range reported by others despite the use of near-tracer additions. As we noted in the Methods, although our additions were at times above the 10% targeted in tracer additions, we do not believe this caused significant rate inflation based on kinetic curves run in parallel. Further, Martin et al. (2012) explicitly investigated the significance of saturating additions with inorganic N in Arctic waters and reported them to be minimal. A companion study that was part of this project found that Urea_N was an important N source during January, especially for prokaryotic populations

(Connelly et al. 2014). The location of the sample sites may have contributed to the relatively high N uptake rates observed in this study. The sites are situated near shore in the Chukchi Sea and are likely influenced by both terrestrial inputs and high nitrate concentrations entering via the Bering Strait inflow (Le Fouest et al. 2013; Tremblay et al. 2015).

In the other studies of the western Arctic, RPIs have indicated low preference for urea even when uptake rates are high (Harrison et al. 1985). Highly variable urea concentrations throughout the region (Simpson et al. 2008) may impact RPI estimates (Stolte and Riegman 1996), as we are only capturing discrete time points. We did not find much difference in ambient urea concentrations between sampling periods (range = $0.14\text{--}0.96 \mu\text{mol N L}^{-1}$; Table 2), and found urea to generally be preferred over NO_3^- , especially in the $> 3.0 \mu\text{m}$ size fraction (Table 5). Even so, rates of urea uptake were exceedingly low ($< 0.2 \text{ nmol N L}^{-1} \text{ h}^{-1}$ during January and April). Only during August, when zooplankton and migrating seabirds are a likely localized source of urea via excretion and sloppy feeding (Conover and Gustavson 1999), does the microbial community respond with increased uptake rates. Urea concentrations were also fairly high during April, most likely due to release from landfast ice (Conover et al. 1999), but uptake rates remained low.

During both the January and April AA_N was the most preferred form of N (Table 5). This is consistent with previous reports from the Arctic that demonstrated that labile DOM constituents, including free amino acids, are an important nutrient resource for prokaryotes (Rich et al. 1997; Yager and Deming 1999; Kirchman et al. 2007; Alonso-Sáez et al. 2008; Nikrad et al. 2012). In marine systems in general, free amino acids can support up to 40% of bacterial production (Kirchman 2000) with prokaryotes able to assimilate them even at nanomolar concentrations (Cottrell and Kirchman 2000; Ouverney and Fuhrman 2000) and subzero temperatures (Yager and Deming 1999). In our study, AA_N uptake was observed during all three sampling periods, with decreased importance relative to other N and C sources only in August. The average molar C : N uptake ratio of the amino acid mixture (i.e., $\text{AA}_\text{C} : \text{AA}_\text{N}$) was 10.5 ± 5.4 for the whole community during January 2012, which is well above the Redfield C : N ratio of 6.6, indicating that C was assimilated preferentially to N. For all other sampling trips it was 2.6 ± 1.0 , indicating that free amino acids were an important source of N. The fact that AA_N was being taken up by the $> 3.0 \mu\text{m}$ size fraction could also be an indication of the importance of DON use by phytoplankton, microflagellates and/or ciliates (Bronk et al. 2007; Sanderson et al. 2008).

In the Arctic, rivers deliver high concentrations of labile organic nutrients (e.g., Dittmar and Kattner 2003; Holmes et al. 2008; McClelland et al. 2012). DON tends to be rapidly consumed in the marine receiving waters, but can also be produced in situ in the Chukchi Sea (Letscher et al. 2013);

this is particularly true of low molecular weight compounds like urea and amino acids. In this study, urea and AA_N were only a small percentage ($< 10\%$) of the overall DON pool, but at times had high rates of uptake compared to inorganic N (see Tables 3, 5). Marine DON consists of a complex mixture of compounds that remain uncharacterized, with urea and free amino acids known to be highly labile (Bronk 2002; Sipler and Bronk 2015). Our results highlight the importance of some fractions of the DON pool, which were likely produced in situ. In the future, the components and lability of Arctic DON is expected to change as more terrigenous material is transported from the tundra to the coastal ocean (Sipler et al. 2017). This highly aromatic terrigenous material will likely be less labile than the low molecular weight compounds studied here, but as of this writing, little is known about the changing bioavailability of DOM entering the Arctic coastal zone.

Importance of regeneration

In August, when NH_4^+ was scarce, we measured relatively high regeneration rates (Table 4), but the relationship between NH_4^+ concentrations and regeneration rates is highly equivocal, with no significant correlation (Fig. 4; $R = 0.39$) between them. While the overall trend appears to be positive, regeneration rates during August seem to generally confound this pattern, such that even though ambient NH_4^+ concentrations are low, regeneration rates are relatively high. This suggests tight coupling with uptake during summer that helps to keep NH_4^+ concentrations low (Clark et al. 2008). During each sample period, the calculated NH_4^+ regeneration rates (including nitrification) were higher than the total combined absolute uptake rate of all the N substrates tested. This implies that the cycling of N within the coastal system is sufficient to supply the N needs of the autotrophic community present.

Nitrification can oxidize a significant portion of NH_4^+ to NO_3^- , even in the surface waters of the Arctic (Simpson et al. 2013b). In the Chukchi Sea, Christman et al. (2011) measured nitrification rates that were 25 times greater during winter (mean = $0.15 \text{ nmol L}^{-1} \text{ h}^{-1}$) than summer (mean = $0.006 \text{ nmol L}^{-1} \text{ h}^{-1}$). We measured significantly higher nitrification rates in our study, which took place in the same region. During the ice-covered periods nitrification ranged from $15.8 \text{ nmol L}^{-1} \text{ h}^{-1}$ to $27.4 \text{ nmol L}^{-1} \text{ h}^{-1}$, and fell to $1.0 \text{ nmol L}^{-1} \text{ h}^{-1}$ in August (Table 4). It is not clear why there is such a great disparity in absolute rates, but the relative rates between sample periods show that nitrification is more active during January as opposed to August. This elevation in nitrification during ice-covered conditions could contribute to the high NO_3^- inventories found generally in the Arctic before the spring bloom period (Tremblay et al. 2008). The fact that nitrification dominates during the low-light sample periods could be a result of light inhibition of nitrification (e.g., Ward 2008), but that relationship is equivocal

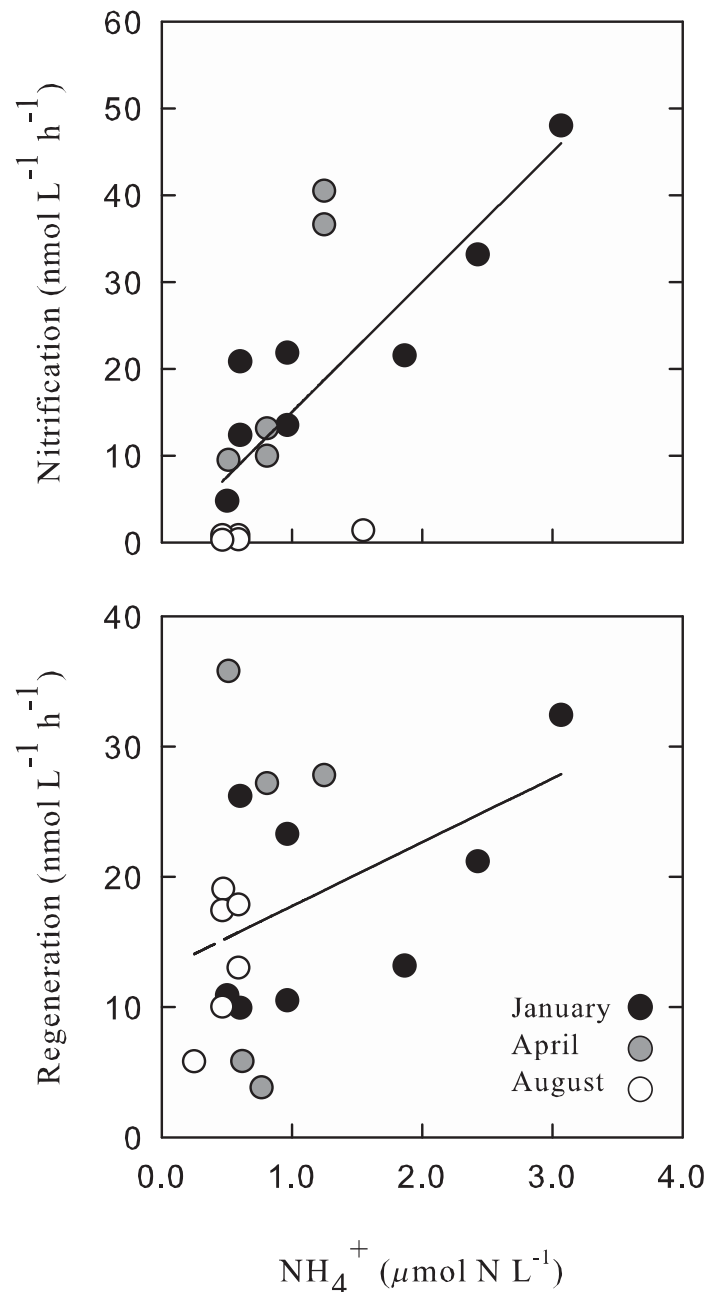


Fig. 4. Ammonium cycling processes. Concentrations of NH_4^+ plotted against rates of nitrification and NH_4^+ regeneration. The regression lines are for all values in the plot (see text for significance and R values).

(Yool et al. 2007). The lack of nitrification in August could alternatively be a function of phytoplankton and bacteria effectively outcompeting nitrifiers for NH_4^+ , which is present at low concentrations. Uptake rates of NH_4^+ were highest in August, which could impact availability of NH_4^+ as a substrate for nitrification. Though nitrification rates were significantly correlated with ambient NH_4^+ concentrations (Fig. 4; $R = 0.67$; $p = 0.0005$), rates of NH_4^+ regeneration were not significantly lower in August relative to January and April. Urea

has also been proposed as a substrate source for nitrification in polar regions (Alonso-Sáez et al. 2012). Although we did not directly measure urea-fueled nitrification rates in our study, potential nitrification rates measured from the NH_4^+ pool were not correlated to urea concentrations ($R = 0.43$; $p > 0.1$).

Conclusions

The western coastal Arctic appears to be increasingly reliant on pelagic processes (Grebmeier et al. 2006). The contribution of the sea ice community to the ecology of the region is sure to decline along with the sea ice habitat. At least in the short term, pelagic phytoplankton will likely respond positively to the earlier light penetration through thinning ice (Lee et al. 2011; Arrigo et al. 2012) and open water conditions (Arrigo et al. 2008). In the future, the Arctic Ocean is also generally expected to be heavily influenced by increased thermal stratification (Peterson et al. 2006). However, the near shore site occupied by our study was never thermally stratified and therefore the physical forcing mechanisms in the shallow coastal areas may respond to other physical processes or not at all. Our work highlights the unique characteristics of the near shore environment of the Chukchi Sea. While there is likely a short-term pulse of NO_3^- uptake that corresponds with the spring bloom period (e.g., Martin et al. 2012), the remainder of the year is heavily dependent on NH_4^+ and organic substrates. The incorporation of seasonal uptake rates of N and C containing compounds is critical for predicting the productivity of the coastal western Arctic. Our research indicates that there is a robust planktonic microbial community during winter that has access to large pools of ambient N, but maintains low levels of assimilation until light limitation is lifted in the summer. The microbial community is able to respond to nutrient availability and utilize multiple forms of both N and C for low levels of growth and maintenance during the ice-covered seasons. We have demonstrated that specific DON components play a large role in the N cycling processes of the coastal western Chukchi Sea. It will be important to understand how future changes in DON delivery and the reduction of sea ice will interact to impact the productivity of this system in the future.

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Conflict of Interest

None declared.

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